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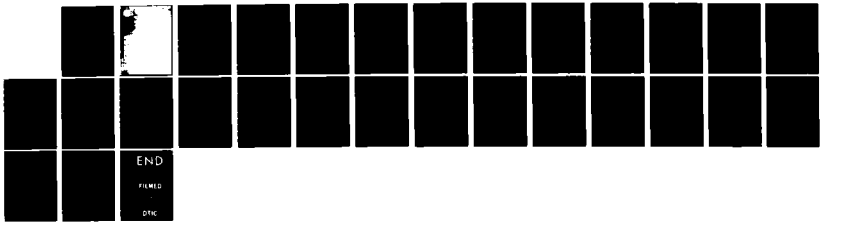

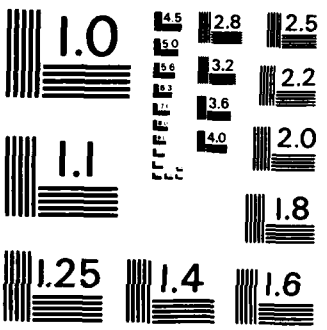

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MONDAY

I. INTRACELLULAR WATER STRUCTURE/TRANSLOCATION

CHAIRPERSON: R. MACELROY (MOUNTAIN VIEW)
FEATURE LECTURE: D. BEVERIDGE (NEW YORK)
DISCUSSION LEADERS: J. CLEGG (CORAL GABLES) AND A. PARSEGIAN (BETHESDA)

J. AND L. CROWE (DAVIS)
W. DROST-HANSEN (MIAMI)
J. FINNEY (LONDON)
N.D. GERSHON (BETHESDA)
S. HOROWITZ (DETROIT)
A. MASTRO (UNIVERSITY PARK)

II. CONCEPTS AND PRINCIPLES OF MEMBRANE PROTON CONDUCTION PATHWAYS

CHAIRPERSON: B. HONIG (NEW YORK)
FEATURE LECTURE: K. SCHULTEN (MUNICH)
DISCUSSION LEADERS: K. SCHULTEN (MUNICH) AND B. HONIG (NEW YORK)

K. DUNKER (PULLMAN)
J. NAGLE (PITTSBURGH)
S. SCHEINER (CARBONDALE)
A. WARSHEL (LOS ANGELES)
G. ZUNDEL (MUNICH)

TUESDAY

III. TRANSLOCATION OF PROTONS/WATER THROUGH LIPOSOMES AND MODEL SYSTEMS

DISCUSSION LEADER: D.W. DEAMER (DAVIS)

D. CAFISO (VIRGINIA)
N. DENCHER (BERLIN)
K. GARLID (TOLEDO)
H. GUTMAN (TEL-AVIV)
R. LAWACZECK (WÜRZBERG)
P. MALONEY (BALTIMORE)
S. MCLAUGHLIN (STONY BROOK)
W. NICHOLS (ATLANTA)
A. PULLMAN (PARIS)

IV. NON BULK PHASE VS. BULK PROTON TRANSLOCATION

DISCUSSION LEADERS: D. KELL (ABERYSTWYTH) AND F. HAROLD (DENVER)

S. FERGUSON (BIRMINGHAM)
J. JACKSON (BIRMINGHAM)
K. HELLINGWERF (NN HAREN, NETHERLANDS)
T.A. KRULWICH (NEW YORK)
B.A. MELANDRI (BOLOGNA)
H. ROTTENBERG (PHILADELPHIA)

WEDNESDAY

V. TRANSLOCATION OF PROTONS THROUGH MEMBRANES: BACTERIORHODOPSIN

CHAIRPERSON: Y. OVCHINNIKOV (MOSCOW)
FEATURE LECTURES: J. LANYI (IRVINE)
D. OESTERHELT (MUNICH): "BACTERIORHODOPSIN AND HALORHODOPSIN: TWO SIDES OF THE RETINAL COIN"

DISCUSSION LEADER: B. HESS (DORTMUND)

E. BAMBERG (FRANKFURT)
L. EISENSTEIN (URBANA)
M. ENGELHARD (DORTMUND)
D. ENGELMAN (NEW HAVEN)
M. HEYN (BERLIN)
D. KUSHMITZ (DORTMUND)
L. KESZTHELYI (SZEGED)
R. MATHIES (BERKELEY)
K. ROTHSCHILD (BOSTON)
L. PACKER (BERKELEY)
K. SCHULTEN (KONSTANZ)
W. STOECKENIUS (SAN FRANCISCO)

VI. TRANSMEMBRANE PROTON TRANSLOCATION BY THE F_0 MOIETY OF H^+-ATP SYNTHASE

DISCUSSION LEADERS: S. PAPA (BARI) AND K.-H. ALTENDORF (ONASBRÜCK)

R. CAPALDI (EUGENE)
F. GUERRIERI (BARI)
J. HOPPE (BRAUNSCHWEIG)
W. JUNGE (USNABRUCK)
E. KLINGENBERG (MÜNCHEN)
R. SANADI (BOSTON)
N. SONE (TOCHIGI-KEN)

THURSDAY

VII. PROTON TRANSLOCATION AND THE CATALYTIC PROCESS IN THE H^+-ATP SYNTHASE

DISCUSSION LEADER: P.D. BOYER (LOS ANGELES)

R. CAPALDI (EUGENE)
C. CARMELI (TEL AVIV)
L. DE MEIS (RIO DE JANEIRO)
L. ERNSTER (STOCKHOLM)
S. FERGUSON (BIRMINGHAM)
W. HUBBELL (LOS ANGELES)
C. KAYALAR (BERKELEY)
H. PENEFSKY (NEW YORK)
H. ROTTENBERG (PHILADELPHIA)

THURSDAY, CONTINUED

VIII. THE FLAGELLAR MOTOR SYSTEM

CHAIRPERSON AND DISCUSSION LEADER: R. MACNAB (NEW HAVEN)
FEATURE LECTURE: H. BERG (PASADENA): "THE FLAGELLAR MOTOR"

FRIDAY

IX. PROTON TRANSLOCATION THROUGH REDOX COMPLEXES

CHAIRPERSON: R. CAPALDI (EUGENE)

FEATURE LECTURE: H.T. WITT (BERLIN): "MOLECULAR MECHANISM OF PROTON GENERATION AND TRANSLOCATION IN PHOTOSYNTHESIS"

DISCUSSION LEADER: M. WIKSTRÖM (HELSINKI)

A. AZZI (BERN)
G.T. BABCOCK (MICHIGAN)
M. BRAND (CAMBRIDGE)
R.P. CASEY (HELSINKI)
S.I. CHAN (PASADENA)
A.R. CROFTS (URBANA)
P.L. DUTTON (PHILADELPHIA)
P.C. HINKLE (ITHACA)
B.G. MALMSTROM (GOTEBORG)
J. NAGLE (PITTSBURGH)
P. RICH (CAMBRIDGE)

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Electrical properties of light driven ion pumps

The electrical properties of the light driven ion pumps Bacteriorhodopsin and Halorhodopsin have been investigated on black lipid membranes.

1. Bacteriorhodopsin

Electrical properties of the photoenergized proton transport in purple membrane (PM) were investigated by measurements of the steady state photocurrent under voltage clamped conditions. Membranes were formed, by depositing in an oriented manner, fragments of PM onto a lipid bilayer support. The ion carrier FCCP and monensin were used to render permeable the lipid bilayer, which is fluid without inducing ion transport in the PM, which is crystalline and therefore remains unaffected by mobile ion carrier. In the dark the conductance of the PM is small, $\leq 10^{-7}$ Scm^{-2}. Illumination of the membrane system increased its conductance up to 5×10^{-5} Scm^{-2}. The purple current voltage curve of the purple membrane indicates that the proton pump behaves within $\pm$ 150 mV as a rectifier.

Noise analysis of the photocurrents demonstrates the occurence of shot noise. From the spectral densitiy at different light intensities, the number of transported charges per absorbed photon could be determined. The result shows that a successful cycle transports two charges, i.e. 2 H^+ per absorbed photon.

2. Halorhodopsin

Membrane fragments, isolated from a Bacteriorhodopsin deficient mutant of Halobacterium halobium and enriched with the chromoprotein HR by treatment with the detergent TWEEN were adsorbed to planar lipid membrane. The photoelectrical properties of HR were studied (ionspecificity and pump activity). To prove, whether the chromoprotein of Halorhodopsin or a complex protein in the bacterial membrane is the functional unit, lipid vesicles reconstituted with the purified protein were attached to planar lipid bilayers and checked on their photoelectrical properties. It was shown, that the chromoprotein alone is the functional unit of Halorhodopsin as the light driven Cl^- pump.

The regulatory effect of blue light was studied on "Tween"-washed membranes and on the proteoliposome planar membrane system. It is demonstrated that green light inactivates the Cl^- pump. Reactivation can be achieved completely by additional blue light.

"The Effect of General Anesthetics on the Relative Permeability of Lipid Bilayers to Protons and Potassium Ions." Gail L. Barchfeld and David W. Deamer, Department of Zoology, University of California, Davis Ca 95616 U.S.A.

The permeability of biological and model membranes to protons is several orders of magnitude greater than that of other ions, possibly due to proton conductance along chains of hydrogen-bonded water molecules within the hydrophobic portion of the bilayer. Bangham et al.(Anesthesiology 53:135, 1980) showed that general anesthetics increase the proton permeability of liposomes and suggested that anesthetics may act by collapsing pH gradients across synaptic vesicle membranes. If anesthetics cause increased amounts of hydrogen-bonded water in the bilayer, then one would expect that the relative permeability to protons would undergo greater increments than potassium permeability as anesthetic concentrations increased. To test this, the proton permeability of liposomes prepared by reverse phase evaporation(egg phosphatidyl choline, egg phosphatidic acid, and cholesterol, 6.5:1:2.5 mole ratio) was measured by monitoring the pH dependent spectral absorbance of 6-carboxyfluorescein encapsulated in the vesicles as buffered pH gradients decayed. Potassium permeability was measured under the same conditions, using a potassium electrode to detect potassium efflux. Our results show that, at clinical anesthetic activities, n-butanol, chloroform, diethyl ether, and halothane produce a two to five fold increment in proton permeability, with a three fold increase for potassium permeability. Relative anesthetic effects on potassium permeability parallel those for proton permeability, suggesting that proton permeability is not specifically increased by anesthetic molecules promoting increased hydrogen-bonded water in the bilayer. Instead, the increased permeability appears to be due to a more general hydrated defect in the bilayer which can be used for passive transport of protons and potassuim ions.

TRANSMEMBRANE ION GRADIENTS IN RECONSTITUTED LIPOSOME SYSTEMS. DETERMINATION OF FUNCTIONAL HETEROGENEITY USING A DUAL-ENTRAPPED PROBE TECHNIQUE.

DUNCAN H. BELL, Department of Botany, University of Glasgow, Glasgow, G12 8QQ, United Kingdom

An assumption of vesicle homogeneity is a common deficiency in approaches to critically resolve enzyme activity in reconstituted vesicle systems. This paper reports on a novel dual entrapped probe method which permits an estimate of the functional heterogeneity in systems which form pH gradients. As a test system, two pH-indicating fluorescent probes were cotrapped in asolectin vesicles during formation. Subsequent reconstitution with bacteriorhodopsin and illumination led to different values for the internal pH reached at steady state. These values were then fit to two simultaneous equations to resolve the degree of functional heterogeneity of the preparation and the true internal pH of the active proteoliposomes.

Such a technique should be useful in studies of other H^+-pumping enzyme systems such as H^+-ATPases and cytochrome oxidase. Additionally, a similar method could be envisioned for determinations of membrane potentials.

No effect of DCCD on the mechanistic $H^+/2e$ ratio of the mitochondrial bc_1 complex

Martin D. Brand, Marwan K. Al-Shawi, Guy C. Brown & Brendan D. Price

Department of Biochemistry, University of Cambridge, U.K.

The force ratio $2\Delta E_h/\Delta p$ across the bc_1 complex approaches the $H^+/2e$ ratio as the complex approaches equilibrium. The $H^+/2e$ ratio can also be determined by steady-state kinetic methods. Under conditions in which dicyclohexylcarbodiimide (DCCD) lowered the estimated $H^+/2e$ ratio from 5.6 to 2.8 (succinate to O_2, kinetic method), the force ratio showed little change, indicating no alteration in the mechanistic $H^+/2e$ ratio of the bc_1 complex. Further experiments eliminate most obvious explanations of the discrepancy between methods, but suggest that DCCD lowers the kinetic estimate of the $H^+/2e$ ratio by a process not involving a change in the mechanistic $H^+/2e$ stoichiometry of the bc_1 complex.

Physical Properties Influencing H^+/OH^- Permeabilities in Phospholipid Vesicles

David Cafiso

Summary:

We have carried out work to elucidate both the source of variability in the reported values for H^+/OH^- permeability and the underlying physical basis for this permeability in phospholipid vesicles. Utilizing spin-labeled hydrophobic ions and ion selective electrodes, proton permeabilities have been characterized for a wide range of vesicle types utilizing a single type of measurement. Not unexpectedly, different vesicle preparations (sonication, reverse phase evaporation, ether injection and detergent dialysis) do yield different values for H^+/OH^- permeability (Pnet). The values vary at most by factors of 10-50, with the sonicated lipid systems being the least permeable. The composition of the lipid utilized has at least as large an effect. In particular, the level of polyunsaturates in phosphatidylcholine has been observed to have very dramatic effects on Pnet. The magnitiude of ΔpH chosen for an experiment alters the measured permeability with larger values of Pnet being obtained for smaller values of ΔpH (as previously observed the size of the pH gradient, $H^+_{in} - H^+_{out}$, also dramatically alters the calculated permeability, indicating that simple diffusion mechanisms cannot alone account for H^+/OH^- currents).

We have completed experiments, aimed at modifying the dipole potential in membrane vesicle systems, to determine the predominant charge carrier for the protonic current. The current appears to be carried largely by a positive species. Several experiments aimed at determining the role of water in the permeation of H^+/OH^- ions have been carried out. In particular, experiments measuring permeabilities in D_2O have been made and two-dimensional NMR experiments that measure cross-relaxation are being utilized to examine water-lipid interactions.

ELECTROGENIC PROCESSES OF THE UBIQUINOL:CYTOCHROME c_2 OXIDOREDUCTASE

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The UQH_2:cyt c_2 O-R complex acts by a modified *Q*-cycle mechanism (1). The complex has three distinct catalytic sites: a quinol oxidase site (the Q_Z-site, or center o); a cyt c_2 reductase site; and a quinone reductase site (the Q_C-site, or center i). Antimycin inhibits the Q_C-site, and prevents the formation of a semiquinone species (Q_C^-) at that site. Myxothiazol and UHDBT (or UHNQ) compete with each other, and with quinol, at the Q_Z-site. The electrogenic process of the complex is associated with the flow of electrons from the Q_Z- to the Q_C-site, through a chain containing two b-cytochrome centers, cyt b-561 (b_H, $E_{m,7}$ 50 mV) and cyt b-566 (b_L, $E_{m,7}$ -90 mV). Net oxidation of 1 mol QH_2/mol complex requires 2 turns over of the Q_Z-site, with the reduction of 2 mol cyt c_2 through a chain containing FeS and cyt c_1, and the delivery of two electrons to the Q_C-site. This is associated with the transfer of 2 moles of charge/mol complex through the electrogenic process (1,2). We have investigated the contributions of different electron transfer reactions to the electrogenic process, by observing the electrochromic carotenoid bandshift (the carotenoid change) under different regimes of inhibition and redox poise.

Antimycin inhibits oxidation of b_H, but not its reduction after a flash, and also inhibits about 80% of the carotenoid change observed with uninhibited chromatophores poised with the quinone pool half-reduced. Further addition of myxothiazol or UHDBT inhibits reduction of b_H (and b_L) and the remaining 20% of the carotenoid change. In the presence of antimycin alone, if b_H is reduced before the flash, reduction of b_L is observed, but no myxothiazol sensitive carotenoid change. These experiments show that both reduction and oxidation of b_H are electrogenic processes, but oxidation of QH_2 by (FeS, c_1,c_2) and b_L is not. By correction for the different stoichiometries of the electrogenic process in the presence and absence of antimycin, it can be shown that reduction of b_H accounts for about 40% of the electrogenic span (3).

When chromatophores are inhibited by myxothiazol, a cyt b-561 can be reduced through the Q_C-site in an antimycin sensitive process (4). This reaction, when initiated by flash illumination, is strongly dependent on pH, with a maximum in the pH range 9.5-11. The dependence on E_h is also of interest, with reduction occuring only when both the quinone pool and b_H are oxidized before the flash. The measured equilibrium constant (K_{eq}) is not consistent with a simple reduction of b_H by quinol from the pool, and we have suggested that the reaction may involve formation of Q_C^- at the site. Under conditions in which the antimycin sensitive reduction of cyt b-561 occurs, the carotenoid change shows an electrogenic process of sign opposite to that of the normal forward reaction. This is taken to indicate that the reaction is a reversal of some part of the normal forward process in which b_H is oxidized (5). Since K_{eq} favors QH_2 oxidation, some other process must contribute to the overall reduction of quinone at this site. We have suggested that a two-electron gate may operate, in which quinone is reduced by sequential 1-electron transfers from b_H (1,2).

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THE CHROMOPHORE RETINAL CONTROLS THE PROTON/HYDROXYL ION FLUX ACROSS BACTERIORHODOPSIN.

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Experiments had been performed to answer the question whether the chromophore retinal in bacteriorhodopsin (BR) influences the proton/hydroxyl ion flux through this light-energized proton pump. Transmembrane pH-gradients were quickly established across BR/BO-dimyristoyl-phosphatidylcholine-phosphatidylserine vesicles and the induced fluorescence changes of the enclosed pH-sensitive optical probe pyranine were measured. The H^+/OH^- flux through bacterioopsin was faster than the one through native bacteriorhodopsin and through bacteriorhodopsin reconstituted from bacterioopsin and retinal. Our results support investigations previously described by Konishi and Packer (FEBS Lett. 89, 333-336, 1978) and might indicate that the chromophore retinal either is part of the proton/hydroxyl ion path across the protein moiety of bacteriorhodopsin or indirectly controls this path. The dependence of the H^+/OH^- permeability on both the isomeric configuration of retinal and the aggregation state of the membrane protein shall be discussed to gain further insight into the molecular properties involved.

On the water permeability across vesicular membranes from binary lecithin mixtures.

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The water permeation across vesicular lecithin bilayers is measured by a new and probe-free technique. The transient technique is based on optical differences of H_2O and D_2O. The experimental quantity is the exchange relaxation rate.

Binary mixtures in excess of water of Dimyristoyl- and Dipalmitoyl-phosphatidylcholine (DMPC:DPPC) and Dimyristoyl- and Distearoyl-phosphatidylcholine (DMPC:DSPC) have been studied. The temperature dependence of the water permeation exhibits a sharp increase at the crystalline to liquid-crystalline phase transition temperature. For binary lecithin mixtures these onset-temperatures coincide with the solidus curves from spectroscopically determined phase diagrams. In the coexistence region the permeation through the already fluid parts or the permeation along the boundaries becomes rate-limiting. The phase diagrams (from onset-temperatures and from spectroscopic studies) are non-ideal but do not show a miscibility gap for the studied mixtures of lecithins which differ by two or four methylene groups in the fatty acyl chains.

PREPARATION AND SPECTROSCOPIC PROPERTIES OF (4-^{13}C-ASP)-bR, (4-^{18}O-TYR)-bR AND (GUANIDO-^{13}C-ARG)-bR

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The photocycle of the purple pigment from Halobacterium Halobium is accompanied by a light driven charge separation[1]. It has been shown by time resolved IR-spectroscopy that three carboxyl-groups (C_1-C_3) are protonated during the rise and decay time of M[2]. Whereas one of these groups (C_1) is deprotonated during the lifetime of L, one of the other (C_3) has a slower kinetic that M[2].

In order to analyse reactive amino acids participating in the pathway of proton transfer we synthesized bacteriorhodopsin with labeled Asp. Incorporation of 4-C^{13}-Asp into the growth medium of Halobacterium Halobium yielded a purple membrane which had an isotope enrichment of 70%. Time resolved IR-experiments showed an isotope shift of 35 cm^{-1} for all three carboxylates. Therefore, these groups can be assigned to three different aspartic acids. Static infrared difference spectroscopy revealed another aspartic acid being deprotonated during the rise time of L. Additionally, it can be shown that one glutamic acid, remaining protonated is perturbated during the photocycle. These results provide considerable constraints on the structural models proposed by Trewhella et al.[3]. The implications of these findings including additional NMR-data will be discussed in regard to the assignment of specific amino acids to the mechanism of the photocycle and proton pump.

Furthermore the preparation of (guanido-^{13}C-Arg)-bR and (4-^{18}O - Tyr)-bR is described and the ^{13}C-solid state NMR and IR-data are discussed.

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DETERMINATION OF THE PROTON-HYDROXYL ION PERMEABILITY THROUGH PHOSPHOLIPID BILAYERS.

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In order to compare the passive proton/hydroxyl ion permeabilities of phospholipid bilayers and of the membrane protein bacteriorhodopsin, H^+/OH^- fluxes across soybean phosphatidylcholine (SBPC) and diphytanoyl PC bilayers were measured. The recently published values of the H^+/OH^- permeability coefficient, Pnet, are quite diverse, ranging from 10^{-3} to 10^{-9} cm/s. In the present study, small unilamellar vesicles formed by sonication from SBPC (average diameter of ~300 Å) or saturated non-oxidizable diphytanoyl PC (diameter ~650 Å) were subjected to fast (<5ms) pH-jumps of 0.2-2.0 units and the response of different pH-sensitive optical probes was monitored. The buffer capacity of the system, i.e. of the buffer, of the dye molecules and of the lipids, was carefully determined. By creating only small pH-gradients and/or by using valinomycin (in the presence of K^+) or lipophilic ions, any influence of a transmembrane diffusion potential was minimized. From experiments with the pH-indicator pyranine entrapped in vesicles values of Pnet ~ 1.5×10^{-4} cm/s for SBPC and Pnet ~ 1.1×10^{-4} cm/s for diphytanoyl PC at 25°C and pH ~ 7 in the presence of 50 mM K_2SO_4 were calculated; the activation energies are 16.6 kcal/mol and 12.6 kcal/mol, respectively. The presence of Cl^- (100 mM) induced a considerable increase in the apparent H^+/OH^- permeability: 1.1×10^{-3} cm/s for SBPC and 2.4×10^{-3} cm/s for diphytanoyl PC. This result indicates that even at neutral pH electroneutral H^+ fluxes can occur by the transmembrane diffusion of molecular HCl. The apparent Pnet determined with the fluorescent amine 9-aminoacridine was about ten times larger than that measured with other pH-probes applied. Control experiments showed that the high H^+/HO^- permeability across SBPC and diphytanoyl PC represents an intrinsic property of these lipid bilayers and is not due to any residual organic solvent (e.g. $CHCl_3$) in the membrane.

THE "ΔpH" PROBE 9-AMINOACRIDINE: RESPONSE TIME AND METHODICAL QUERY.

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In a great number of publications during the past years the magnitude of the proton gradient across biological and artificial membranes has been determined by means of the fluorescent amine 9-aminoacridine (9-AA). The quantitative evaluation of the experimental data was based on the following assumptions: the uncharged form of 9-AA is freely permeable across the membrane, the charged species does not permeate, 9-AA is not bound significantly to the membrane, in the presence of a pH-gradient 9-AA is concentrated in the internal water phase (Vi) where it loses completely its fluorescence (1). In order to test the validity of these crucial assumptions, and to determine the response time of this method and the parameters which influence it, transmembrane pH gradients were established in about 5 ms by utilizing a rapid-mixing stopped-flow spectrofluorometer, and the induced fluorescence changes of 9-AA were recorded. A variety of pure lipid and reconstituted protein-lipid vesicles were examined. Some of the experimental results obtained challenge the application of 9-AA as accurate monitor of ΔpH-changes, e.g.:

1. *in the absence of a pH-gradient* 9-AA fluorescence changes occurred upon energization of the system (2);
2. in certain systems, in which a large pH-gradient was present, no fluorescence quenching of 9-AA could be observed;
3. the apparent decay rate of the pH-gradient established was about ten times faster when monitored with 9-AA as compared to other pH-probes applied.

These observations and our important finding that negatively charged membrane constituents are a necessary prerequisite for the energy-dependent 9-AA fluorescence quenching do not agree with the proposed reaction mechanism (1) for this dye. We shall discuss alternative models. Furthermore, data for the dependency of the 9-AA response time on the physical and chemical state of the membrane are presented.

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(2) N.A. Dencher, Biophys. J. 41 (1983) 372a.

ROLE OF POLYPEPTIDES AND OF AMINOACID RESIDUES OF THE MITOCHONDRIAL H^+-ATPase COMPLEX IN THE H^+-TRANSLOCATION FUNCTION

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The mitochondrial H^+-ATPase complex consists of three subunits: the hydrophilic F_1 sector, the proton translocating sector F_o, and the "stalk" which binds F_1 to F_o and seems to play a role for the gating of the proton current during its functional coupling to the catalytic activity in F_1 (1).

The F_o sector is an oligomeric protein which, in addition to the well known 8 kDa polypeptide directly involved in H^+ conduction (2), contains, at least, other 4 polypeptides (3,4) i.e.: 31 kDa, two in the 20-25 kDa region and Factor 6. It is not known if these polypeptides are directly involved in the H^+ translocation or if they have only a structural role.

The stalk contains the OSCP and probably Factor B (3).

Studies with aminoacid modifiers on H^+-conduction by the F_oF_1 complex, in submitochondrial particles with various degree of resolution of the complex, have revealed that, in addition to the well known glutamic residue of the 8 kDa polypeptides, other aminoacid residues in the F_o and "stalk" are involved in H^+-conduction (3,5-8). In particular two classes of aminaocid residues can be identified: 1) The aminoacids whose modification results in inhibition of H^+ conduction; 2) the aminoacids whose modification results in stimulation of H^+ conduction. In the first case the aminaocid modifiers inhibit proton conduction in all the types of submitochondrial particles tested and also in the F_o reconstituted liposomes. Results are presented which indicate that the aminoacids modified are located in the membrane in structural and/or functional association with the critical glutamic residue of the 8 kDa polypeptide. Results with the second type of aminoacid modifiers indicate that polar aminoacid residues located superficially in the F_o sector or in the stalk polypeptides are involved in the gate and coupling function of the complex.

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CHROMOPHORE STRUCTURE IN DARK-ADAPTED BACTERIORHODOPSIN DETERMINED BY SOLID-STATE NMR

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The Department of Physiology and Biophysics, Harvard Medical School, Boston, Massachusetts, the Department of Chemistry, University of California, Berkeley, California, the Department of Chemistry, Rijksuniversiteit te Leiden, Leiden, The Netherlands, and the Francis Bitter National Magnet Laboratory, Massachusettts Institute of Technology, Cambridge, Massachusetts.

NMR spectroscopy, using cross polarization and magic angle spinning combined with specific isotopic labelling, is a powerful non-perturbative means of probing specific aspects of membrane protein structure. Using these techniques, we have determined the complete disposition of the conjugated polyene chain of dark-adapted bacteriorhodopsin (bR). Initially, ^{15}N-NMR of ϵ-^{15}N-lysyl-bR showed the Schiff base linkage to be protonated and weakly hydrogen bonded to its counterion. ^{13}C-NMR of white membranes reconstituted with specifically labelled retinals has proven to be equally useful, being highly sensitive to chain configuration. For example, ^{13}C-12-retinyl-bR shows a wide separation between the resonance positions of the 13-cis and all-trans isomers of dark-adapted bR, attributed to the strong steric effect at C-12 in the 13-cis isomer. A similar effect at C-14 was used to infer for the first time a syn C=N configuration in the 13-cis isomer. The second kink in the chain compensates for the first and allows the 13-cis,15-syn isomer to fit the same binding site as the all-trans,15-anti isomer. More recently, we have shown via model compound studies that the ^{13}C chemical shift at C-5 is very sensitive to the conformation of the 6-7 bond. The correspondingly labelled bR shows the chromophore to be in the relatively unusual 6-s-trans form. ^{13}C-NMR also provides supporting evidence of a point charge at C-5 and suggests an additional positive charge near C-7 and C-19, possibly an arginine.

Altered Solvent Properties of Nucleoplasmic Water. S.B. Horowitz and D.S. Miller, Department of Physiology and Biophysics, Michigan Cancer Foundation, Detroit, MI 48201.

We measured the equilibrium partition coefficients (EPC) of 18 solutes between nuclear gel in situ and a gelatin gel internal reference phase (iRP) whose solvent properties were those of ordinary water. Most solutes (amino acids, polyols, sodium, ATP, thymine, thymidine, sucrose and other glycosides) distributed with nucleus/iRP EPC of unity. These solutes experience nuclear gel water and ordinary water as identical solvents. In contrast, sugars which contain a hemiacetal configuration of hydrogen bonding groups (3-O-methyl-D-glucose, L-glucose, 2-deoxy-D-glucose, D-xylose and D-ribose) had nucleus/iRP EPC 20-30% below unity. These monosaccharides experience nuclear gel water as a poorer solvent than ordinary water.

To explain reduced monosaccharide solvency, we have adopted the model that (i) ordinary water consist of transient ("instantaneous") hydrogen-bonded microstructures whose shapes require a statistical description and are influenced by the presence of non-water molecules; (ii) that hemiacetal monosaccharides "fit" the microstructures of ordinary water especially well and, therefore, have excess solubility in this media. Reduced monosaccharide solvency implies that the average "instantaneous" structure of nuclear gel water is different from ordinary water, presumably because of interactions with the macromolecular cytomatrix. Supported by NIH grants GM19548 and an institutional grant from the United Foundation of Greater Detroit.

Abstract for Poster presentation at the Gordon Research Conference on "Protons and Membrane Reactions", Santa Barbara - January 1985

Proton Translocation coupled to electron transport in Photosynthetic bacteria

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Factors which affect the "backlash" in proton uptake by chromatophore membranes at the onset of illumination include:

(1) Backlash in the electron transport chain. A burst of electron transport through the photosynthetic reaction centre and associated proton translocation takes place before steady-state cycling of electrons through the cytochrome $\underline{b}/\underline{c_1}$ complex is reached.

(2) Limitation of electron transport by membrane potential. The electron transport rate is shown to depend sensitively on $\Delta\psi$. As $\Delta\psi$ rises at the onset of illumination, electron transport is progressively inhibited.

(3) The non-ohmic proton conductance of the membrane. Proton efflux from the chromatophores increases disproportionately as $\Delta\psi$ rises at the onset of illumination.

Rapid H^+ efflux, observed in intact cells at the onset of illumination, is more complex than in chromatophores, although the data are also consistent with proton translocation being coupled to electron transport. After short flashes the half-time for H^+ release measured with cresol red is about 35 ms. This reaction is abolished by myxathiazol but inhibited only about 40% by antimycin. The data are consistent with the H^+-release accompanying the oxidation of ubiquinol by the Rieske FeS and cytochrome $\underline{b}_{566}$ at centre Q_z.

EFFECTS OF THIOCYANATE AND VENTURICIDIN ON RESPIRATION-DRIVEN H^+ TRANSLOCATION IN *PARACOCCUS DENITRIFICANS*

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1. A fast-responding O_2 electrode [1] has been used to confirm and extend [2] observations [3,4] of a significant kinetic discrepancy between O_2 reduction and consequent H^+ translocation in "O_2-pulse" experiments in intact cells of *P.denitrificans*. The chaotropic SCN^- ion abolishes this discrepancy, and greatly increases the observable $\rightarrow H^+/O$ ratio, to a value approaching its accepted, true, limiting stoichiometry. The observable H^+ decay rates are very slow, particularly in the absence of SCN^-.

2. The submaximal $\rightarrow H^+/O$ ratios observed in the absence of SCN^- are essentially independent of the size of the O_2 pulse when this is varied between 4.7 and 47 ng atom, in a manner not easily explained by a delocalised chemiosmotic energy coupling scheme.

3. Osmotically active protoplasts of *P. denitrificans* do not show a significant kinetic discrepancy between O_2 reduction and H^+ ejection, even in the absence of SCN^-. However, the submaximal $\rightarrow H^+/O$ ratios observed in the absence of SCN^- are again essentially independent of the size of the O_2 pulse. As in intact cells, the observable H^+ decay rates are extremely slow.

4. The energy transfer inhibitor venturicidin causes a significant increase in the $\rightarrow H^+/O$ ratio observed in *P. denitrificans* protoplasts in the absence of SCN^-; the decay kinetics are also somewhat modified. Nevertheless, the $\rightarrow H^+/O$ ratio observed in the presence of venturicidin is also independent of the size of the O_2 pulse in the above range. This observation militates further against arguments in which (a) a non-ohmic backflow ("leak") of H^+ from the bulk aqueous phase might alone be the cause of the low $\rightarrow H^+/O$ ratios observed in the absence of SCN^-, and (b) in which there might be a Δp-dependent change ("redox slip") in the actual $\rightarrow H^+/O$ ratio.

5. It is concluded that the observable protonmotive activity of the respiratory chain of *P. denitrificans* in the absence of SCN^- is directly influenced by the state of the H^+-ATP synthase in the cytoplasmic membrane of this organism. We are unable to explain the data in terms of a model in which the putative protonmotive force may be acting to affect the $\rightarrow H^+/O$ ratio.

6. One possibility, which would conveniently serve to explain these and other [5] data, is that the bulk-to-bulk phase membrane potential set up in response to protonmotive activity is energetically insignificant. Since the apparent membrane potential, as judged by steady-state ion uptake measurements, is insensitive to respiration rate over a wide range [6], one should predict that the kinetics of ion uptake (in a chemiosmotic model) would be similarly insensitive to respiration rate. Such an experimental test might allow one to distinguish the veracity of "localised" [7] and "delocalised" energy coupling models in electron transport phosphorylation [8].

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DCCD-sensitive ATPase in Halobacterium saccharovorum.

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ABSTRACT

Membranes from Halobacterium saccharovorum contained a cryptic ATPase which required Mg^{2+} or Mn^{2+} and was activated by Triton X-100. The optimal pH for ATP hydrolysis was 9-10. ATP or GTP were hydrolyzed at the same rate while ITP, CTP, and UTP were hydrolyzed at about half that rate. The products of ATP hydrolysis were ADP and phosphate. The ATPase required high concentrations (3.5 M) of NaCl for maximum activity. The apparent K_m for ATP was 4 mM. ADP was a competitive inhibitor of the activity, with an apparent K_i of 0.05 mM. Dicyclohexylcarbodiimide (DCCD) inhibited ATP hydrolysis. The inhibition was marginal at the optimum pH of the enzyme. When the ATPase was preincubated with DCCD at varying pH values, but assayed at the optimal pH for activity, the DCCD inhibition was observed to increase with increasing acidity of the preincubation medium. DCCD inhibition was also dependent on time of preincubation, and protein and DCCD concentration. When preincubated at pH 6.0 for 4 h at a protein:DCCD ratio of 40 (w/w), ATPase activity was inhibited 90%.

ABSTRACT

UNCOUPLER-INDUCED ATP SYNTHESIS IN THE METHANOGENIC BACTERIA. J.R.Lancaster,jr., B.P.Crider,K.R.Rogers,and S.W.Carper, Department of Chemistry and Biochemistry, Utah State University, Logan,UT.

The methanogenic bacteria are strictly anaerobic archaebacter, most of which are capable of the eight-electron reduction of CO_2 by H_2 to form CH_4 and H_2O. The fact that many species can grow completely autotrophically on these gaseous substrates in the absence of any added carbon compound has been used to rule out substrate-level phosphorylation. The recent apparent elimination of intracellular membranes ("methanochondria") as involved in methane formation leaves the cytoplasmic membrane as the most likely site for a chemiosmotic mechanism, which has been generally assumed. The detection of cellular ion gradients has been cited as support for such a conclusion. We report here that:

1.The addition of high concentrations (5 uM) of the potent uncoupler SF6847 or of valinomycin plus external potassium has no effect on either methanogenesis or ATP synthesis driven by this electron transfer in cells of M. voltae.
2.ATP synthesis in cells can be driven by a negative membrane potential, induced by valinomycin addition in the absence of added potassium(internal K concentration has been reported to be >0.7 M in this species); this synthesis is not eliminated by uncoupler.
3.ATP synthesis is also induced by a base to acid transition, in the presence of valinomycin plus external potassium. This synthesis is eliminated by uncoupler.
4.Synthesis of ATP can also be induced by an acid to base transition, but only in the presence of uncoupler. Valinomycin plus external potassium eliminates this synthesis.

Our interpretation of these results is that ATP synthesis coupled to electron transfer in methanogens does not involve the obligatory intermediacy of a transmembrane ion gradient, but the cells possess an ion-translocating ATPase (not involving either protons or potassium) responsible for the maintenance of intracellular ion composition.

DCCD binding to Subunit VIII of the mitochondrial bc_1 complex inhibits H^+ translocation

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Studies from our laboratory have shown that DCCD at low concentrations inhibits proton translocation in bc_1 proteoliposomes (1,2).Under the same conditions ^{14}C-DCCD binds only to Subunit VIII of the complex,and a good correlation is found between the time course of binding to Subunit VIII and inhibition of proton translocation,whereas the much slower binding to cytochrome b is correlated with inhibition of electron transfer. We are now characterizing the properties of isolated Subunit VIII in liposomes(3).

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ANION EXCHANGE IN BACTERIA: PROTON-LINKED VARIABLE STOICHIOMETRY FOR PHOSPHATE:SUGAR 6-PHOSPHATE ANTIPORT IN STREPTOCOCCUS LACTIS

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Streptococcus lactis expresses anion exchange that uses monovalent phosphate (Pi) to support an electroneutral antiport with those sugar 6-phosphates (S6Ps) that appear as intracellular products of bacterial phosphotransferase (pts) enzyme II^{man}. This specificity suggests that exchange can direct a metabolic regulation by balancing pts-mediated input with chemiosmotic efflux.

Work discussed here has examined the effect of pH on the exchange ratio for phosphate and 2-deoxyglucose 6-phosphate antiport. Membrane vesicles were made to contain 50 mM KPi and 10 mM $MgSO_4$ (pH 7) and then suspended at pH 5.2, 6.1 or 7 in 100 mM KCl, 60 mM Na_2SO_4, 20 mM MOPS (or MES)-K, with 0.5 µM valinomycin and 0.25 mM Na_3VO_4. During a 30 min preload, vesicles were exposed to either ^{14}C-labeled sugar phosphate or ^{32}Pi. Stoichiometry was measured in the next phase, when internal label was chased by addition of an excess of the alternate substrate (^{32}Pi or ^{14}C-labeled sugar phosphate). For experiments at pH 7, an exchange of 2:1 (Pi:S6P) was observed, as exected for the neutral antiport of 2 Pi monoanions and 1 divalent S6P. At lower pH, the coupling ratio fell, to 1.5:1 at pH 6.1 and 1.1:1 at pH 5.2, and this suggests an electroneutral character is preserved by exchange of one or two monovalent Pi anions with a single mono- or divalent S6P. Such behavior is compatible with a reaction mechanism that requires simultaneous binding of internal and external substrates. The role of H+ would be indirect, in that variable stoichiometry arises to compensate for protonation of a substrate, not of the protein itself.

ROLE OF POLYPEPTIDES IN THE PROTONMOTIVE ACTIVITY OF CYTOCHROME c REDUCTASE OF MITOCHONDRIA

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Results will be presented on the role of oloproteins of the b-c_1 complex in the proton-motive activity of the cytochrome c reductase.

Studies with chemical modifiers of aminoacid residues and digestion with specific proteolytic enzymes will be reported.

Evidence is presented for an involvement of core protein II and low molecular weigth oloprotein (14, 8 e 7 kDa) in proton translocation.

In the model we favour polypeptide subunits are proposed to constitute pathways for transmembrane proton conduction from the matrix aqueous phase to a semiquinone quinol system in the membrane and from this to the opposite outer phase.

TRANSPORT OF PROTONS ACROSS MEMBRANES BY UNCOUPLERS OF OXIDATIVE PHOSPHORYLATION

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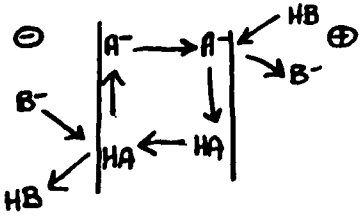
The mechanism by which the weak acids FCCP, CCCP and S-13 transport protons across phospholipid bilayer membranes is now well understood. The neutral, HA, and charged, A^-, forms of these weak acids adsorb strongly to membrane-solution interfaces by means of hydrophobic interactions. The π electrons delocalize the charge on the anion, lowering the Born energy required to partition A^- from water (dielectric constant 80) into the interior of the membrane (dielectric constant 2). Thus A^- is soluble in the interior of the membrane and the bilayer is permeable to A^-. The results of our kinetic voltage-clamp and charge-pulse experiments and the laser-induced proton jump experiments of Gutman and his colleagues are consistent with the following model. When a voltage of the sign indicated by the encircled + and - in the figure below is applied to the membrane, the A^- species moves from the left-hand interface to the right-hand interface. The increase in concentration at the right hand interface causes a buffer molecule from the aqueous phase, HB, to combine with and donate a proton to A^-, forming HA. HA moves down its concentration gradient, from the right- to the left-hand interface, where it combines with the B^- form of the aqueous buffer to yield A^-. The protons are transferred through the aqueous unstirred layers adjacent to the membrane by the "buffer shuttle" mechanism. Surface charges, surface dipoles and the dielectric constant of the interior of the membrane all have the expected effects on the carrier-mediated transport of protons.

The ability of weak acids to uncouple oxidation from phosphorylation in mitochondria can be understood in terms of their protonophore activity. This observation cannot, however, be used to distinguish between local and bulk *chemiosmotic schemes*: the high concentration of protonophores at the membrane-solution interface might allow them to dissipate a localized as well as a bulk proton gradient.



PROTON-TRANSLOCATING PROPERTIES OF MITOCHONDRIAL NICOTINAMIDE NUCLEOTIDE TRANSHYDROGENASE FROM BEEF HEART.

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Mitochondrial nicotinamide nucleotide transhydrogenase catalyzes the reversible transfer of hydride ion equivalents between NADH and $NADP^+$ and the concomittant translocation of protons across the mitochondrial inner membrane (1-3). Being a proton pump the transhydrogenase is kinetically and thermodynamically coupled to the prevailing proton motive force of the membrane (2). Proton-translocation apparently occurs through a DCCD-sensitive proton channel (4,5) at a proton/hydride ion ratio of 0.5-1.0 (6,7) and is catalyzed by a dimeric enzyme (8). The fact that beef heart transhydrogenase is composed of only a single polypeptide of a molecular weight of about 115,000 which is reconstitutively active, i.e. it can be incorporated in a liposomal membrane and generate an uncoupler-sensitive membrane potential (9) and proton gradient (6), suggests that this enzyme may be the most suitable model system available for studies of the mechanism of redox-driven proton pumps.

Although beef heart transhydrogenase earlier has been purified to apparent homogeneity by several different methods (10-14), the use of new and highly sensitive protein-staining methods have revealed minor but unidentified and possibly important contaminating peptides in the preparations obtained by earlier methods. In search of a procedure suitable for a highly reproducible preparation of large amounts of homogeneous enzyme, a recently developed procedure including fast protein liquid chromatography (FPLC) was utilized yielding a preparation reconstitutively active which catalyzes a membrane proton-translocation and the generation of a pH-gradient as judged by quenching of 9-aminoacridine fluorescence (15).

An important step in the developed procedure is the removal of residual Triton X-100 which was shown to block proton-translocation unless carefully removed by lowering the Triton X-100 binding capacity of the enzyme by washing the preparation with 0.5% sodium cholate prior to reconstitution. Furthermore the pure transhydrogenase isolated by the FPLC procedure is strongly activated by addition of a cholate-phospholipid suspension suggesting that Triton X-100 has the capacity of replacing bound phospholipids and thereby afflicting the proton-translocation as well as the catalytic activity of the enzyme possibly due to a structural rearrangement of the native enzyme.

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MECHANISM WHICH DELAYS THE PROPAGATION OF pH-PULSES ALONG THE PARTITION REGION OF STACKED THYLAKOIDS

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When the proton pumps in thylakoids are stimulated by light flashes protons are taken up from the suspending medium. This, however, is very much delayed (100ms) as compared with the reduction of electron acceptors at both photosystems (1ms). With cresol red as pH-indicator we found different mechanisms for the delay, depending on which photosystem was driving proton consumption. In the presence of methyl viologen as Hill acceptor the alkalization caused by photosystem I activity was slow because of the slow dismutation of formed superoxyde. The alkalization caused by photosystem II, on the other hand, was dependent on the stacking conditions. It was accelerated by the unstacking of thylakoids and it was decelerated upon their restacking.

Therefore, the observed delay was not mainly due to proteinaceous diffusion barriers for protons but for photosystem II, it was probably caused by multiple binding and release of protons in the highly buffered partition region, as previously proposed (Hong S & W Junge (1983) Biochim Biophys Acta 722, 197-205). It is calculated that this delay mechanism in the propagation of a proton pulse would neither impede a high rate nor a high efficiency of photophosphorylation under steady illumination.

Abstract for Gordon Conference Contribution

Activation Energies for Proton Channels in Bacteriorhodopsin

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The temperature dependence of the photovoltage developed by a model membrane containing bacteriorhodopsin was measured. The time course of the photovoltage in response to a laser flash was measured over the range of 1 μs to several seconds at temperatures from 1°C to 40°C. The photoresponse is taken to be a sum of exponentials. Exponential time constants and amplitudes are determined by an analysis of the photoresponse using 3 different techniques: a photovoltage versus log time plot, a correlation filter and non-linear least squares. The photovoltage is taken to be the sum of 3 exponentials but only two of the three time constants are resolved. Both of these time constants are temperature dependent and indicate a thermally activated transport process. The corresponding activation energies are 55 kJ/mole and 62 kJ/mole. Since the photovoltage is proportional to charge times displacement the corresponding charge displacements are 11 $\mathring{A}$ and 34 $\mathring{A}$ assuming a total displacement of 45 $\mathring{A}$. The remaining exponential term in the photovoltage corresponds to a small negative transient that has a rise time less than 1 μs even at -20°C. The corresponding charge displacement is estimated to be less than 2 $\mathring{A}$.

CHARACTERISATION OF PURPLE AND WHITE MEMBRANES IN SUSPENSION USING QUASI-ELASTIC LIGHT SCATTERING AND ELECTRON MICROSCOPY

A.E. Robinson, E. Hrabeta, L. Packer, B. Arrio, G. Johannin, P. Volfin, and M. Lefort-Tran

Quasi-elastic laser light scattering was used to characterize the size of isolated purple and white membrane preparations before and after proteolysis. Chemically modified purple membrane preparations were also analyzed. The results show that native purple membrane preparations have a larger average size than would be expected, and become even larger following trypsin treatment. White membranes, however, exhibit an average size consistant with that reported from electron microscopy. After retinal reconstitution or trypsin treatment, the size of the white membrane preparations remains unchanged; however, bleaching and retinal reconstitution of purple membranes increases the size of the preparations. Negative staining electron microscopy was used to show that purple membranes aggregate in stacked arrays. In aggregated purple membrane preparations, protons and other cations are unable to exchange freely with the aqueous medium, which explains why proteolysis lowers the proton-pumping efficiency of purple membranes in suspension. Reports of proton-pumping effeciency restoration in proteolyzed preparations upon liposomal reconstitution may indicate that aggregation is reduced by this procedure.

COUPLING OF CONFORMATIONAL CHANGES IN A PROTEIN TO PROTON TRANSFERS BETWEEN RESIDUES

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Ab initio molecular orbital calculations have been carried out to determine mechanisms whereby conformational changes within a protein molecule could be directly coupled to transfers of protons from one group to another. It is found that motions of an ion in the vicinity of a H-bond can drastically alter the energetics of proton transfer within that bond. It is possible for a cation to "push" a proton across from one group to another with a normally lower pK. The effect of this ion is not damped by a shielding of the proton from its field by the system but is instead magnified by a factor of nearly two. A second mechanism for shifting a proton from A to B is by means of a change in the angular features of the A·H··B bond. In general, bending of group B relative to the A--B axis preferentially stabilizes configuration A··HB over AH··B; i.e. it pulls the proton across toward group B. This effect is strong enough to draw a proton from a carbonyl to a hydroxyl which normally has a lower pK. Similar observations of the imine-amine pair lead to proposition of a simple mechanism whereby the light-induced motion of the Schiff base in bacteriorhodopsin could result in a proton transfer to a neighboring H-bonded group.

Abstract

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The ATP synthase (F_1F_o) of Escherichia coli is associated with the cytoplasmic membrane and consists of two structurally and functionally distinct entities: the peripheral F_1 portion which carries the catalytic centers of the enzyme and the integral F_o complex which translocates protons across the membrane (1-3).

The F_o complex, which is built up from three kinds of subunits a, b and c with the proposed stoichiometry of $1:2:10_{\pm 1}$, has been purified in an active form and in high yield (4). We have dissociated the F_o complex by treatment with trichloroacetate (3 M) at pH 8.0, in the presence of deoxycholate (1%) and N-tetradecyl-N, N-dimethyl-3-ammonio 1-propanesulfonate (Zwittergent 3-14, 5%). The subunits were separated by gel filtration with trichloroacetate (1 M) included in the elution buffer. The homogeneity of the fractions was checked by re-chromatography and SDS-gel electrophoresis. After integration into phospholipid vesicles each subunit alone as well as all possible combinations were tested for H^+ translocating activity and binding of F_1. A functional H^+ channel could only be reconstituted by the combination $a_1b_2c_{10}$ which corresponds to that of native F_o (5,6).

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The Effect of Protonation and Coulomb Interactions on the Bond Structure of Retinal Schiff Bases and Related Compounds

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Protonation thoroughly changes the electronic structure of conjugated molecules, in particular, the height of ground state isomerization barriers of π-bonds. Due to the high polarizability of their π- system the stereochemical properties of the protonated molecules are very sensitive to the actual distribution of charges in the surroundings. This behavior has been demonstrated by quantum-chemical MNDOC calculations for several retinal Schiff base analogues and for stilbazolium betaines.

MNDOC is today the most sophisticated and computationally extensive semiempirical all-valence electron method. The necessary computations for the calculation of equilibrium geometries and adiabatic isomerization potentials with all essential geometric degrees of freedom optimized for molecules as large as retinal were possible only using a CRAY-1 computer and vectorized algorithms.

In the case of the retinal Schiff base the double bond most strongly affected by protonation of the terminal nitrogen is the 13 14 bond. Protonation reduces its isomerization barrier from 47 kcal/mol to 11.5 kcal/mol. This latter barrier can be shifted further between 37 kcal/mol and 6 kcal/mol when two negative charges are suitably positioned in the neighbourhood. Similarly, but less drastically, the barrier can be altered in the range between 16 kcal/mol and 4 kcal/mol upon replacing the 13 methyl group by residues like -H, C_2H_5, $-CF_3$ or $-OCH_3$. The results explain the proton pumping activities of bacterio-opsin reconstituted with these analogues and elucidate the decisive role of retinal's ground state intramolecular properties in the pump kcycles of bacteriorhodopsin and halorhodopsin.

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Biological Transport of Protons

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General: We have studied in quantitative detail a model for the biological transport of protons /1/. This model purports that protons are conducted through transmembrane proteins by means of a linear chain of hydrogen-bridges formed by molecules of bound water and the amino acid side groups. For such a conductor we have investigated the time dependence of the following observables: 1) proton current, 2) charge displacement within the conductor, 3) free energy decrement, 4) state of protonation of the conductor groups. These observables were determined for the following possible experiments: the measurement of the pH-dependence of the stationary, voltage-induced proton current; the coupling of the proton transport to either alternating electric fields or electric field jumps; the response of the conductor to the injection or ejection of a proton. Each of the above-mentioned experiments should give, as our calculations have shown, certain information about the elementary steps and the groups involved in the proton transport.

Proton Channel in ATP-ase: An important step on the way to understanding the transport of protons in biological systems is the recent measurement of the conductance of single proton channels in the proteolipids of ATP-ase. We derive a simple analytical expression for the conductance of a single proton channel in terms of the pK values of the conducting groups and the kinetic constants of the elementary steps and compare the results to the measured conductance values/2/. This comparision revealed that the observed conductance is in agreement with a model of a proton channel constructed from bound water and indicated that the hydrogen bonds between the groups are weak, i.e. can be broken on a time scale of 100 ps.

Blue Light Effect in Bacteriorhodopsin: To model the proton transport in bacteriorhodopsin we have connected a proton channel to an acid or a base with the capability to inject a proton into (or eject a proton from) the conductor. Allowing the interaction between the proton conductors and injecting or ejecting groups to be time-dependent, we investigated the refractory phase that exists after an intial proton current pulse and demonstrated the buffering capacity of the conductors, a function that we associate with the 'blue light effect' of bacteriorhodopsin.

Proton Diode: Our calculations have demonstrated that by choosing conductor groups with different pK values or through the interaction with an internal electric field, a proton conductor with the voltage-current characteristics of a diode can be realized.

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DETERMINATION OF RETINAL CHROMOPHORE STRUCTURE IN BACTERIORHODOPSIN AND HALORHODOPSIN WITH RESONANCE RAMAN SPECTROSCOPY

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Resonance Raman spectra have been obtained of bacteriorhodopsin and its photointermediates using purple membrane regenerated with ^{13}C- and deuterium-labeled retinals. Selective isotopic substitution permits the assignment of the Raman lines to specific vibrational normal modes, while model compounds and vibrational calculations have been used to develop simple rules for determining chromophore structure from these assignments. These methods have been used to determine the cis/trans configuration about the C_{13}=C_{14} and C=NH bonds in bacteriorhodopsin's intermediates. The chromophore structure in the acidified and deionized forms of bacteriorhodopsin, and in halorhodopsin, the chloride ion pump of H. halobium, has also been examined. These results are summarized below:

(1) The configuration about the C_{15}=NH Schiff base bond can be determined from the extent of coupling between the NH in-plane rock and the C_{14}-C_{15} stretch.[1] The rock-stretch coupling is large when the C=N configuration is cis resulting in a 40-60 cm^{-1} shift of the C_{14}-C_{15} stretch upon deuteration of the Schiff base nitrogen. Conversely, the rock-stretch coupling is small in the C=N trans geometry, producing only a slight (<5 cm^{-1}) shift of the C_{14}-C_{15} stretch upon N-deuteration. Based on the sensitivity of the C-C stretches to N-deuteration, we have determined that the C=N configuration is cis in BR_{548} and trans in BR_{568}. Thus, dark-adaptation involves a concerted "bicycle pedal" isomerization about both the C_{13}=C_{14} and C=N bonds which produces no large changes in the Schiff base environment. Conversely, because both BR_{568} and K_{610} have C=N trans chromophores, the BR_{568} → K_{610} transition involves a single isomerization about the C_{13}=C_{14} bond which translates the Schiff base proton into a new protein environment facilitating vectorial proton transfer.

(2) Resonance Raman spectra of acidified (pH 2) and deionized bacteriorhodopsin (denoted BR^{A}_{605} and BR^{D}_{605}, respectively) are very similar, and they bear a close correspondence with the Raman spectrum of dark-adapted bacteriorhodopsin which contains an approximately equal mixture of 13-cis and all-trans retinal protonated Schiff base chromophores.[2] The close similarity of the BR^{A}_{605} and BR^{D}_{605} spectra suggests that the formation of each pigment results from displacement of a divalent cation from its protein binding site. The Raman spectrum of BR^{A}_{565}, the pH 0 form of bacteriorhodopsin, is nearly identical to that of BR_{568}, demonstrating that BR^{A}_{565} contains an all-trans protonated Schiff base chromophore. The close similarity between BR^{A}_{565} and BR_{568} suggests that the effects produced by lowering the pH to 2 are reversed at pH 0.

(3) We have recently published resonance Raman spectra of the hR_{578} form of halorhodopsin.[3] The close similarity of the frequencies and intensities of the hR_{578} Raman bands with those of BR_{568} shows that the chromophore in hR_{578} has an all-trans configuration and that the protein environment around the chromophore in these two pigments is very similar. Presumably, this means that hR_{578} experiences an electrostatic perturbation near the ionone ring as does BR_{568}. In addition, hR_{578} exhibits a Raman line at 1633 cm^{-1} which is assigned as the stretching vibration of a protonated Schiff base linkage to the protein based on its shift to 1627 cm^{-1} in D_2O. The unusually weak N-H rock to C=N stretch coupling (6 cm^{-1}) may result from altered H-bonding to an electronegative group in the protein.

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ABSTRACT

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The role of δ and ε in F_1 from E. coli has been studied using isolated native and chemically modified subunits. In reconstitution experiments with membrane vesicles and 3-and 4-subunit F_1, the effect of modifying thiol and histidine groups on ATPase activitiy, binding of F_1, and ATP-driven energy-dependent processes has been investigated.

HOW TO CORRELATE THE STRUCTURE AND EFFICIENCY OF LIGHT INDUCED PROTON PUMPS

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ABSTRACT

The key factors that control the efficiency of light induced proton transfer across membranes are considered. It is shown that the kinetics of the pumping process is determined by the energetics of the intermediate proton transfer states. It is demonstrated how to use microscopic and macroscopic electrostatic concepts to determine the relevant free energy barriers. The approach presented allows one to correlate the efficiency of proton pumps with their actual structure and to predict the effects of electric fields, external charges, and genetic modifications.

APPARENT DIRECT COUPLING IMPLIED BY SMALLNESS OF CHEMIOSMOTIC COUPLING UNITS

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The proton space for membrane linked free-energy transduction may be much smaller than the entire mitochondrial matrix space [review: 1]. In fact, current models suggest that the effective coupling unit does not involve more than a few electron-transfer chains and H^+-ATPases. One implication is that the pumping of some two protons into the implied proton domain would already induce synthesis of ATP. Since the relative magnitude of fluctuations is on the order of $1/\sqrt{N}$, this implies large fluctuations in the local proton concentration: the customary enzyme kinetic and thermodynamic approaches may break down.

Using statistical mechanical approaches that are insensitive to the magnitudes of fluctuations, we asked whether this phenomenon would also result in failure of the generally accepted implications of chemiosmotic coupling schemes [2]. We report that this is indeed so: In a model where electron-transfer linked proton pump, H^+-ATPase and proton leak consist of mechanically independent enzymatic cycles, we find that: (1) the (average) rate of ATP synthesis is not a unique function of the (average) proton gradient (plus properties of the H^+-ATPase); it matters whether one varies the leak or the activity of the electron-transfer chain. (2) ATP synthesis may take place even though the local proton 'potential' is thermodynamically incompetent with respect to the free-energy of ATP hydrolysis.

Consequently, if the protons are accepted to be restricted to small domains, much experimental data can be understood in terms of chemiosmotic coupling [2].

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